

CLAIMS

1. A method for controlling the production of cellular (3R)-hydroxyacyl CoA esters of predetermined length in a host cell or organism, c h a r a c t e r i z e d in that the method

comprises the following steps:

- introducing a gene encoding a multifunctional 2-enoyl-CoA hydratase 2/(3R)-hydroxyacyl CoA dehydrogenase enzyme type 2 protein (MFE-2) comprising at least one gene region encoding a hydratase domain and at least one gene region encoding a dehydrogenase domain, and wherein at least one genetic change has been made to the gene region encoding dehydrogenase domain, resulting in the enrichment of cellular (3R)-hydroxyacyl CoA esters of predetermined length, when the gene is introduced and expressed in a host cell oxidizing exogenous or endogenous fatty acids, or a DNA construct or a vector comprising the gene, into a host cell or organism; and

-growing the host cell or organism under suitable growth conditions in the presence of a carbon source comprising fatty acids from an endogenous source or originating from exogenous additions, resulting in the enrichment of cellular (3R)-hydroxyacyl CoA esters of predetermined length.

2. A method for preparing a host cell or organism capable of producing PHAs, said host cell or organism expressing an endogenous or foreign gene or genes encoding (3R)-hydroxyacyl-CoA ester polymerizing enzyme or enzymes, c h a r a c t e r i z e d in that the method comprises the following steps:

- introducing a gene encoding a multifunctional 2-enoyl-CoA hydratase 2/(3R)-hydroxyacyl CoA dehydrogenase enzyme type 2 protein (MFE-2), which comprises at least one gene region encoding a hydratase domain and at least one gene region encoding a dehydrogenase domain, wherein at least one genetic change has been made to the gene region encoding dehydrogenase domain, resulting in the enrichment of cellular (3R)-hydroxyacyl CoA esters of predetermined length, when the gene is introduced and expressed in a host cell oxidizing exogenous or endogenous fatty acids, or a DNA construct or a vector comprising the gene, into the host cell or organism; and

-growing the host cell or organism under suitable growth conditions in the presence of a carbon source comprising fatty acids from an endogenous source or originating from exogenous additions.

3. A method for producing PHAs in a host cell or organism expressing endogenous or foreign gene or genes encoding (3R)-hydroxyacyl-CoA ester polymerizing enzyme or enzymes, characterized in that the method comprises the following steps:

- introducing a gene encoding a multifunctional 2-enoyl-CoA hydratase 2/(3R)-

5 hydroxyacyl CoA dehydrogenase enzyme type 2 protein (MFE-2) comprising at least one gene region encoding a hydratase domain and at least one gene region encoding a dehydrogenase domain, wherein at least one genetic change has been made to the gene region encoding dehydrogenase domain, resulting in the enrichment of cellular (3R)-hydroxyacyl CoA esters of predetermined length, when the gene is introduced and
10 expressed in a host cell oxidizing exogenous or endogenous fatty acids or a DNA construct or a vector comprising the gene, into a host cell or organism;

-growing the host cell or organism under suitable growth conditions in the presence of a carbon source comprising fatty acids from an endogenous source or originating from exogenous additions; and

15 -recovering the PHAs or their hydrolysis products.

4. The method according to any one of claims 1-3, characterized in that the gene encoding the endogenous MFE-2 is inactivated in the host cell or organism.

20 5. The method according to any one of claims 1-3, characterized in that the gene encoding MFE-2 originates from yeast.

6. The method according to any one of claims 1-3, characterized in that the genetic change comprises inactivation of the gene region encoding the domain responsible for the
25 oxidation of cellular short chain length (3R)-hydroxyacyl CoA esters resulting in the enrichment of cellular (3R)-hydroxyacyl CoA esters of C4-C8 chain length.

7. The method according to any one of claims 1-3, characterized in that the genetic change comprises inactivation of the gene region encoding the domain responsible for the
30 oxidation of cellular (3R)-hydroxyacyl CoA esters of C8-C16 chain length resulting in the enrichment of cellular (3R)-hydroxyacyl CoA esters of C8-C16 chain length.

8. The method according to any one of claims 1-3, characterized in that the gene originates from mammals.

9. The method according to claim 8, characterized in that the change comprises inactivation of the gene region encoding the dehydrogenase domain resulting in the accumulation of cellular (3R)-hydroxyacyl CoA esters of C8 - C18 chain length.

5

10. The method according to claim any one of claims 1-3, characterized in that the gene originates from yeast which encodes a multifunctional 2-enoyl-CoA hydratase 2/(3R)-hydroxyacyl CoA dehydrogenase enzyme type 2 protein comprising one gene region encoding hydratase domain and two gene regions encoding dehydrogenase domains, and wherein both gene regions encoding dehydrogenase domains are inactivated resulting in the enrichment of cellular (3R)-hydroxyacyl CoA esters of about C4 – C20 length, when the gene is introduced and expressed in a host cell capable of β -oxidation of fatty acids.

10

15

11. A host cell or organism according to any one of claims 1-3, characterized in that the host cell is a bacterial, a yeast, a fungus or a plant cell.

12. The host cell or organism according to claim 11, characterized in that the host cell is a plant cell and that the method comprises growing the transformed plant cell into a transgenic plant.

20

13. A host cell or organism selected from the group consisting of a bacterial, a yeast, a fungus or a plant cell, characterized in that the host cell or organism is prepared by the method of claim 2.

25

14. A host cell or organism according to claim 13, characterized in that it is a plant cell, a plant or its progeny.

30

15. A gene encoding a multifunctional 2-enoyl-CoA hydratase 2/(3R)-hydroxyacyl CoA dehydrogenase enzyme type 2 protein (MFE-2) comprising at least one gene region encoding a hydratase domain and at least one gene region encoding a dehydrogenase domain,

characterized in that at least one genetic change has been made to the gene region encoding dehydrogenase domain, resulting in the enrichment of cellular (3R)-hydroxyacyl CoA esters of predetermined length, when the gene is introduced and expressed in a host cell oxidizing exogenous or endogenous fatty acids.

5

16. The gene according to claim 15, characterized in that the gene originates from yeast.

10

17. The gene according to claim 16, characterized in that the genetic change comprises inactivation of the gene region encoding the domain responsible for the oxidation of cellular short chain length (3R)-hydroxyacyl CoA esters resulting in the enrichment of cellular (3R)-hydroxyacyl CoA esters of C4-C8 chain length.

15

18. The gene according to claim 17, characterized in that the gene encodes the amino acid sequence of SEQ ID NO. 17 or 21.

20

19. The gene according to claim 16, characterized in that the genetic change comprises inactivation of the gene region encoding the domain responsible for the oxidation of cellular (3R)-hydroxyacyl CoA esters of C8-C16 chain length resulting in the enrichment of cellular (3R)-hydroxyacyl CoA esters of C8-C16 chain length.

20. The gene according to claim 19, characterized in that the gene encodes the amino acid sequence of SEQ ID NO. 16 or 20.

25

21. The gene according to claim 15, characterized in that the gene originates from mammals.

30

22. The gene according to claim 21, characterized in that the genetic change comprises inactivation of the gene region encoding the dehydrogenase domain resulting in the accumulation of cellular (3R)-hydroxyacyl CoA esters of C8 - C18.

23. A yeast gene encoding a multifunctional 2-enoyl-CoA hydratase 2/(3R)-hydroxyacyl CoA dehydrogenase enzyme type 2 protein comprising one gene region encoding hydratase domain and two gene regions encoding dehydrogenase domains, characterized

z e d in that both gene regions encoding dehydrogenase domains are inactivated resulting in the enrichment of cellular (3R)-hydroxyacyl CoA esters of about C4 – C20 length, when the gene is introduced and expressed in a host cell capable of β -oxidation of fatty acids.

5

24. The gene according to claim 23, c h a r a c t e r i z e d in that the gene encodes the amino acid sequence of SEQ ID NO. 18 or 22.

25. A DNA construct comprising the gene of claim 15.

10

26. A vector comprising the gene of claim 15.

27. A host cell comprising the gene of claim 15.

15

28. The host cell of claim 27, c h a r a c t e r i z e d in that the cell is a bacterial, yeast, fungus or a plant cell.

29. A plant or its progeny comprising the cells of claim 28.

20

30. A multifunctional enzyme encoded by the gene of claim 15.

31. A method for preparing a host cell or organism capable of expressing a modified 2-enoyl-CoA hydratase 2/(3R)-hydroxyacyl CoA dehydrogenase enzyme type 2 protein, c h a r a c t e r i z e d in that the method comprises the following steps:

25

- introducing the gene of claim 15 into a host cell or organism; and
- growing the host cell under suitable conditions.

32. A host cell or organism prepared by the method of claim 31, c h a r a c t e r i z e d in that it is selected from the group consisting of host cells or organisms belonging to bacteria, yeast, fungus or plants.

30